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10/804,339	03/19/2004	Patricia Cruz-Perez	0001-00001CON1	8012
7590 Patricia Cruz, Ph.D. Harry Reid Center for Environmental Studies 4505 Maryland Parkway Box 454009 Las Vegas, NV 89154-4009				
EXAMINER				
WOOLWINE, SAMUEL C				
ART UNIT		PAPER NUMBER		
1637				
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

### Office Action Summary

**Application No.**

10/804,339

**Applicant(s)**

CRUZ-PEREZ ET AL.

**Examiner**

SAMUEL WOOLWINE

**Art Unit**

1637

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --  
**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 24 August 2007.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 18-27 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 18-27 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SE/US)  
Paper No(s)/Mail Date \_\_\_\_\_
- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date \_\_\_\_\_
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_

## DETAILED ACTION

### Status

Claims 18-27 are pending in the application. Upon further consideration and consultation, the finality of the Office action mailed 08/14/2006 is withdrawn in consideration of the finding that the language of claim 18, specifically "obtaining DNA standards" and "comparing amplification plots...of each of the DNA standards" implies multiple standards, whereas Haugland used only one standard (i.e. a "single calibrator sample"; page 330, column 1, 2<sup>nd</sup> paragraph). Hence subtracting the  $C_T$  of this standard from the  $C_T$  of the target does not suffice for "comparing amplification plots...of each of the DNA standards". Therefore, all previous rejection are withdrawn and new rejections are set forth below. Accordingly, this action is NON-FINAL.

Arguments made in the brief submitted 08/24/2007 will be addressed to the extent that such arguments apply to the new rejections. Note the rejections set forth below do not rely on *In re Deuel*, which the examiner agrees does not bear on the issues at hand.

### Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 18 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Haugland et al (Molecular and Cellular Probes 13:329-340 (1999), cited on the IDS of 03/19/2004) in view of Heid et al (Genome Research 6:986-994 (1996), cited on the IDS of 03/19/2004).

With regard to claim 18, Haugland teaches a method comprising:

obtaining a primer set and probe that is specific for the fungal species

Stachybotrys chartarum; See page 334, first sentence of *Results*: "The sequences and target sites of the forward (StacF4) and reverse (StacR5) PCR primers and TaqMan probe (StacP2) constructed for the detection of *S. Chartarum* rDNA sequences in this study are shown in Fig. 1." See also figure 1.

collecting the sample from the environment; See page 333, first sentence of *Collection, recovery and analysis of conidia from air samples*: "Air sampling was performed in rooms that had previously been occupied by infants diagnosed with PH from three homes in the Cleveland, Ohio area."

extracting the sample's DNA; See page 334, last sentence of first paragraph on the page: "Three additional 10µl aliquots of each recovered sample were mixed with G.

*candidum* reference conidia and subjected to total genomic DNA extraction for subsequent analysis in the model 7700 as specified above.”

obtaining DNA standards from a culture of *Stachybotrys chartarum*; See page 330, first sentence of second paragraph under *Fungal cultures, conidia stocks and genomic DNA extraction*: “Genomic DNAs were extracted from 20µl conidia suspensions using a glass bead milling and glass milk adsorption method.”

determining the concentration of *Stachybotrys chartarum* spores in the DNA standards; See page 330, fourth sentence of first paragraph under *Fungal cultures, conidia stocks and genomic DNA extraction*: “Cell concentrations in these stock suspensions were determined by counting under a microscope at 400× magnification in a haemocytometer chamber, after which the suspensions were divided into ~200µl aliquots for storage at -80°C.”

amplifying by polymerase chain reaction each of the DNA standards and the collected sample's DNA using the obtained primer set and probe; See page 332, *PCR amplification and TaqMan analysis in the model 7700*, entire section.

With regard to claim 21, wherein the concentration of *Stachybotrys chartarum* spores in the DNA standards is determined via direct total count of the *Stachybotrys chartarum* spores in the DNA standards, Haugland teaches on page 330, fourth sentence of first paragraph under *Fungal cultures, conidia stocks and genomic DNA extraction*: “Cell concentrations in these stock suspensions were determined by counting under a microscope at 400× magnification in a haemocytometer chamber, after which the suspensions were divided into ~200µl aliquots for storage at -80°C.”

Haugland does not teach comparing amplification plots obtained by polymerase chain reaction of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *Stachybotrys chartarum* in the collected sample and a concentration of the fungus *Stachybotrys chartarum* in the collected sample. Rather, Haugland determines presence and quantity of the fungus by comparing the  $C_T$  value of the test sample with the  $C_T$  value of a single standard (i.e. calibrator; see page 330, column 1, 2<sup>nd</sup> paragraph).

Heid demonstrates it was well-known in the art to quantify a target by comparing the  $C_T$  value obtained for a sample containing that target to a standard curve constructed by plotting the  $C_T$  values versus standard concentration for a series of standards of known concentrations. For example, at page 991, paragraph bridging columns 1-2, Heid describes an experiment in which the quantity of factor VIII plasmid DNA (pF8TM) in transfected cell samples "was determined by extrapolation to the x-axis of the standard curve in Figure 4B". This appears to be the same basic concept illustrated in Applicant's figure 8. In the absence of any evidence to the contrary, this is how the examiner has construed the limitation comparing amplification plots obtained by polymerase chain reaction of each of the DNA standards and the collected sample's DNA.

Hence, it would have been *prima facie* obvious to one of ordinary skill in the art to modify the method taught by Haugland for detecting/quantitating *S. chartarum* by using the standards obtained by Haugland to construct a standard curve, and determining the concentration of *S. chartarum* collected samples based on this. It would have been

obvious because this was a well established practice in real-time quantitative PCR technology as evidenced by Heid. Furthermore, Haugland himself alludes to such a practice at page 330, column 1, 2<sup>nd</sup> paragraph: "Thus far, such quantitative results have been obtained most commonly through the use of external standard curves" (citation omitted). Although Haugland discusses here advantages to the comparative C<sub>T</sub> method he uses ("eliminates the time and reagent consuming process of generating standard curves"), this does not make the claimed methods any less obvious. An old technique is not patentable simply because a newer, perhaps easier or faster technique has emerged.

Claims 19-20 and 22-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Haugland et al (Molecular and Cellular Probes 13:329-340 (1999), cited on the IDS of 03/19/2004) in view of Heid et al (Genome Research 6:986-994 (1996), cited on the IDS of 03/19/2004) as applied to claims 18 and 21 above, and further in view of GenBank GI:3420911 [online] June 11, 1999 [retrieved on 06/19/2008] retrieved from <http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=3420911> (prior art of record), and Buck et al (BioTechniques 27(3):528-536, September 1999, prior art of record).

The teachings of Haugland and Heid have been discussed.

The only limitations of claims 19-20 and 22-27 not taught by Haugland and Heid are the specific primers/probes (SEQ ID NOS 1-5) used for the quantification of *Stachybotrys chartarum*. SEQ ID NOS 1-5 were all known sequences of *Stachybotrys*

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*chartarum* at the time the invention of the instant application was made as shown by

GenBank GI:3420911.

## SEQ ID NO 1

```
>F|g13420911:gb|AF081469.1:AF081469 Stachybotrys chartarum strain UAMH 7900 28S ribosomal RNA gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene, partial sequence
Length=936

Score = 34.2 bits (17), Expect = 2e-05
Identities = 17/17 (100%), Gaps = 0/17 (0%)
Strand=Plus/Plus

Query 1 GTTGCCTCGCGCGGAAC 17
      |||
Subject 405 GTTGCCTCGCGCGGAAC 421
```

## SEQ ID NO 2

```
>F|g13420911:gb|AF081469.1:AF081469 Stachybotrys chartarum strain UAMH 7900 28S ribosomal RNA gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene, partial sequence
Length=936

Score = 40.1 bits (20), Expect = 5e-07
Identities = 20/20 (100%), Gaps = 0/20 (0%)
Strand=Plus/Minus

Query 1 TTTCGCTTCCACACAGAG 20
      |||
Subject 511 TTTCGCTTCCACACAGAG 492
```

## SEQ ID NO 3

```
>F|g13420911:gb|AF081469.1:AF081469 Stachybotrys chartarum strain UAMH 7900 18S ribosomal RNA gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene, partial sequence
Length=936

Score = 38.2 bits (19), Expect = 2e-06
Identities = 19/19 (100%), Gaps = 0/19 (0%)
Strand=Plus/Plus

Query 1 ACCATACGTTGCTTCGGG 19
      |||
Subject 458 ACCATACGTTGCTTCGGG 416
```

## SEQ ID NO 4



art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents." Buck provides the rationale as to why one of ordinary skill in the art would have regarded all possible subsequences of a known sequence as equivalents for the purpose of serving as primers, as well as probes, for that known sequence (after all, a primer must be able to hybridize specifically and be extended by a polymerase, whereas a probe need only hybridize specifically). Furthermore, Buck provided a reasonable expectation of substituting the claimed primers and probes for the ones used by Haugland. Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95

control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Since the rejection is based on the conclusion that it would have been obvious to one of ordinary skill in the art to make and use the primers and probes of the claimed methods, being functionally equivalent to those used by Haugland, the limitation "wherein the forward primer, reverse primer and probe do not cross-react with other fungal species when used in combination in polymerase chain reaction" would necessarily have been met.

#### ***Response to Arguments***

With regard to claims 18 and 21, Applicant's brief filed 08/24/2007 set forth two limitations of claim 18 allegedly not taught in Haugland: (i) "obtaining a primer set and probe that is specific for the fungal species *Stachybotrys chartarum*" and (ii) "comparing amplification plots obtained by polymerase chain reaction of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *Stachybotrys chartarum* in the collected sample and a concentration of the fungus *Stachybotrys chartarum* in the collected sample" (page 7 of brief). With regard to the second item, this argument is moot in view of the new grounds of rejection.

With regard to the first item, more precisely, Applicant has argued that the primers and probes used by Haugland are not "specific" for *Stachybotrys chartarum* (page 7 of brief, emphasis in original). This is based on the fact that Haugland's forward primer (StacF4) is completely homologous to the rDNA sequences of *S. dichroa*, *M.*

*echinata* and *M. subsimplex* (page 8 of Brief and Haugland FIG. 1). To support the argument, Applicant also cites page 334, column 1, second paragraph of Haugland: "Priming efficiency analysis indicated that the rDNA sequences of two of these species, *S. cylindrospora* and *S. oenanthes*, had the potential to be amplified by both StacF4 and StacR5."

It is to be noted, however, that there is no explicit definition of the word "specific" in Applicant's disclosure, and there is certainly no requirement that a particular primer or probe sequence is found nowhere else in nature for the primer or probe to be considered "specific" for *Stachybotrys chartarum*.

Furthermore, claim 18 reads: "a primer set and probe that is specific for the fungal species *Stachybotrys chartarum*". Therefore, this can be construed as requiring only a probe that is specific for this species. Alternatively, this could be construed as requiring that the *combination* of the primers and probe are specific for this species. With this in mind, and in response to Applicant's citation of Haugland, it is important to consider Haugland's very next statement (page 334, column 1, second paragraph): "Mismatch Tm analyses suggested, however, that the sequences of both of these [other] organisms would be discriminated against by the StacP2 probe at the standard 60°C annealing temperature employed by the model 7700 in Taqman analyses." Hence, Haugland's probe, and indeed the combination of primer set and probe, was specific for the fungal species *Stachybotrys chartarum*. That this was the case is supported by several statements in the Haugland reference:

"In this study, real-time polymerase chain reaction (PCR) product analysis using the TaqMan™ fluorogenic probe system and an Applied Biosystems Prism® model 7700 sequence detection instrument (model 7700) was applied to the specific detection of *S. chartarum*." (abstract, emphasis provided)

"In the present study, this sequence information was applied toward the development of a PCR assay for the specific detection of *S. chartarum* rDNA..." (page 330, column 1, first paragraph, emphasis provided)

"Experimental testing of the specificity of the probe and primer set in the model 7700 was performed using  $5 \times 10^2$  purified rDNA templates from each of these [other] organisms. In these experiments, the mean  $C_T$  value of three replicate reactions containing *S. chartarum* templates was determined to be 23.7, SD: 0.5. In contrast, no significant reporter dye fluorescence was detected in any of the reactions containing templates from the other organisms during the 40 amplification cycles performed by the instrument." (page 334, column 1, last paragraph, emphasis provided)

Therefore, Haugland meets the limitation of "obtaining a primer set and probe that is specific for the fungal species *Stachybotrys chartarum*".

With regard to claims 19-20 and 22-27 and Applicant's arguments on pages 10-12 of the brief, it is not disputed that the primers recited in the claims and those recited in Haugland represent different portions of known sequences of *Stachybotrys chartarum*. The examiner agrees with Applicant's treatment of *In re Deuel*, and therefore the current rejections do not rely on this case. Nevertheless, one having

ordinary skill in the art of nucleic acid amplification, e.g. PCR, would have recognized that the claimed primer sequences would have represented obvious equivalents to those taught in Haugland for the purpose of specific detection of this organism in view of the known sequence disclosed in GenBank GI:3420911 and the disclosure of Buck et al.

Applicant disputes this position on page 14 of the brief, where Applicant argues that such equivalency "must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents" (emphasis in original). Applicant concedes that the primers of the present application are functionally equivalent to the primers of Haugland (brief, page 14), but disputes that such would have been recognized in the prior art. The examiner disagrees, noting that one of ordinary skill in the art, reading the disclosure of Buck et al, would have realized that any subsequence of a known sequence would have had a reasonable expectation of success in functioning as a primer for that known sequence.

Applicant argues on page 15 of the brief (emphasis provided) that "the mere disclosure of a genomic sequence does not offer any information as to regions that are unique for amplifying a specific target organism. One skilled in the art will recognize that primers from any given sequence, such as GENBANK, must be carefully designed and extensively tested to verify that the designed primers actually detect only the target organism". On this point, the examiner agrees. While any subsequence of a known sequence would have had a reasonable expectation of being used successfully as a

primer of that known sequence, one of skill would have considered specificity when practicing the method of Haugland. As Applicant has stated, one skilled in the art would have recognized this. In fact, Haugland himself taught this (see page 331, "Design and synthesis of TaqMan probe and primer sets" and see page 334, column 1, last paragraph). Hence, one of ordinary skill in the art would have been well aware of the need for specificity when practicing Haugland's method, and could have easily arrived at alternative primers and probes (including the claimed primers and probes) based on known sequences through routine experimentation (e.g. consideration of "corresponding rDNA sequences of non-target organisms" as discussed by Haugland at page 331, column 2, or through "[e]xperimental testing of the specificity of the probe and primer set" as discussed by Haugland at page 334, column 1, last paragraph).

Finally, Applicant argues that Buck would not have provided a reasonable expectation of success in "achieving the claimed invention". Presumably this means a reasonable expectation of success in substituting the claimed primers for those used by Haugland. The examiner fails to see why this is the case, since virtually ever primer designed to the known sequence in Buck functioned adequately as a primer. With regard to achieving specificity (i.e. of detecting only the desired organism), this issue has already been discussed in the immediately preceding paragraph.

In summation, one having ordinary skill in the art would have recognized from Buck's disclosure that any subsequence of a known sequence would have had a reasonable expectation of success in being used as a primer for that sequence. Hence, the disclosure of the *Stachybotrys chartarum* sequence in GenBank GI:3420911 would

have rendered any subsequence thereof (including the claimed sequences) *prima facie* obvious for use as primers or probes. One of skill in the art would also have been aware of the need to maintain specificity based on Haugland's disclosure and as conceded by Applicant on page 15 of the brief. Thus one of skill in the art would have recognized Applicant's primers and probes to represent not more than equivalents of those used by Haugland for the purpose of specific detection of *S. chartarum*.

With regard to the declaration provided by Dr. Stetzenbach, it is noted that she does not agree that the cited references provide a reasonable expectation of success, presumably on the assertion that "[e]xtensive research and validation" would have to be conducted to design primers and probes that are specific (page 3 of declaration). Firstly, the disclosure of Haugland shows that this was within the level of skill of the ordinary artisan. Secondly, the elements listed as "extensive research" on pages 18-19 of the brief, while representing investment of time, labor and expense, are nevertheless nothing more than routine experimentation as disclosed in Haugland (e.g. consideration of "corresponding rDNA sequences of non-target organisms" as discussed by Haugland at page 331, column 2, or through "[e]xperimental testing of the specificity of the probe and primer set" as discussed by Haugland at page 334, column 1, last paragraph).

Hence, the subject claims are obvious over the disclosures of Haugland, Heid, Buck and GenBank GI:3420911.

***Conclusion***

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/  
Examiner, Art Unit 1637

/GARY BENZION/  
Supervisory Patent Examiner, Art Unit 1637